

AMENDMENTS TO THE CLAIMS

This listing replaces all prior versions and listings of claims in the application.

Listing of Claims

Claim 1 (Previously presented) A method for the preparation of a recombinant polypeptide comprising

a) transforming a non-human host cell with an expression vector comprising:

(1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to

(2) a chimeric nucleic acid sequence that encodes a fusion protein, wherein said chimeric nucleic acid sequence comprises (a) a nucleic acid sequence encoding a full-length chymosin pro-peptide, linked in reading frame to (b) a nucleic acid sequence that is heterologous to the pro-peptide and that encodes the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide; operatively linked to

(3) a nucleic acid sequence encoding a termination region that is functional in said host cell,

b) growing the non-human host cell to produce said fusion protein,

c) obtaining said fusion protein from said non-human host cell, and

d) contacting said fusion protein with a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide, whereby said chymosin pro-peptide is cleaved from said fusion protein to release said recombinant polypeptide.

Claims 2-3 (Canceled).

Claim 4 (Currently amended) The method according to claim 1 wherein said aspartic protease of step e) d) is selected from the group consisting of chymosin, pepsin, pepsinogen, cathepsin and yeast proteinase A.

Claim 5 (Previously presented) The method according to claim 1 wherein the recombinant polypeptide is hirudin or carp growth hormone.

Claim 6 (Previously presented) The method according to claim 1 wherein the chimeric nucleic acid sequence does not include a sequence encoding a mature form of chymosin.

Claim 7 (Previously presented) The method according to claim 1 wherein step d) is effected at a pH of from about 2 to about 7.

Claim 8 (Previously presented) The method according to claim 7 wherein the pH is from about 2 to about 4.5.

Claim 9 (Currently amended) The method according to claim 1 wherein step d) is effected ~~under~~ in vitro ~~conditions~~.

Claim 10 (Currently amended) The method according to claim 1 wherein step d) is effected ~~under~~ in vivo ~~conditions~~.

Claim 11 (Canceled).

Claim 12 (Previously presented) The method according to claim 10 wherein step d) is effected in the milk, the stomach, or the gut of an animal.

Claim 13 (Previously presented) The method according to claim 1 wherein the aspartic protease of step d) is chymosin.

Claim 14 (Previously presented) The method according to claim 1 wherein the aspartic protease of step d) is heterologous to the chymosin pro-peptide.

Claim 15 (Currently amended) The method according to claim 13 wherein step d) is effected ~~under~~ in vitro ~~conditions~~.

Claim 16 (Currently amended) The method according to claim 13 wherein step d) is effected ~~under~~ in vivo ~~conditions~~.

Claim 17 (Canceled).

Claim 18 (Previously presented) The method according to claim 16 wherein step d) is effected in the stomach, gut, or milk of an animal.

Claim 19 (Previously presented) The method according to claim 1 wherein said nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

Claims 20-47 (Canceled).

Claim 48 (Currently amended) A method for the preparation of a recombinant polypeptide comprising

a) transforming a non-human host cell with an expression vector comprising:

(1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to

(2) a chimeric nucleic acid sequence that encodes a fusion protein, wherein said chimeric nucleic acid sequence comprises (a) a nucleic acid sequence encoding a full-length chymosin pro-peptide, linked in reading frame to (b) a nucleic acid sequence that is heterologous to the pro-peptide and that encodes the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide; operatively linked to

(3) a nucleic acid sequence encoding a termination region that is functional in said host cell,

b) growing the non-human host cell to produce said fusion protein, and
c) contacting said fusion protein with a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide, whereby said chymosin pro-peptide is cleaved from said fusion protein to release said recombinant polypeptide,

~~The method of claim 10~~ wherein step d) c) is effected by expressing said aspartic protease in said host cell.

Claim 49 (Currently amended) The method of claim 46 48 wherein the aspartic protease of step c) is chymosin and wherein step d) c) is effected in vivo by expressing said aspartic protease in said host cell.

Claim 50 (Previously presented) The method according to claim 1 wherein said aspartic protease of step d) is pepsin.

Claim 51 (Previously presented) A method for the preparation of a recombinant polypeptide, comprising

a) transforming a host cell with an expression vector comprising:

(1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to

(2) a chimeric nucleic acid sequence that encodes a fusion protein, wherein said chimeric nucleic acid sequence comprises (a) a nucleic acid sequence encoding a full length chymosin pro-peptide, linked in reading frame to (b) a nucleic acid sequence that is heterologous to the pro-peptide and that encodes the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide; operatively linked to

(3) a nucleic acid sequence encoding a termination region that is functional in said host cell,

wherein the host cell is selected from the group consisting of bacterial cells, yeast cells and plant cells,

- b) growing the host cell to produce said fusion protein;
- c) contacting said fusion protein in vivo with a mature form of an autocatalytically maturing aspartic protease that cleaves the pro-peptide by expressing said autocatalytically maturing aspartic protease in said host cell,
whereby said pro-peptide is cleaved from said fusion protein to release said recombinant polypeptide.